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Aggregative behaviour in *Folsomia candida* (Collembola: Isotomidae), with respect to previous conditioning

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With one figure

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1. Introduction

Aggregated distribution patterns have been observed in many species of Collembola, including *Folsomia candida* (Willem), both in the field (Glasgow 1939; Joosse 1970; Usher 1969), and in the laboratory (Verhoef & Nagelkerke 1977; Usher & Hider 1975; Verhoef *et al.* 1977).

Usher & Hider (1975) noticed that aggregations of *F. candida* only occurred if sufficient individuals were present, and increased in size as more animals are added. It was also noted that the relative size of any aggregation increased with time. These findings suggest some mutual attraction between the animals. Verhoef *et al.* (1977) suggested that the aggregative response in three species of Entomobryidae was due to a pheromone deposited by the animals. Collembola, therefore, tended to aggregate in areas previously occupied by members of the same species.

This paper reports investigations into the aggregation of *F. candida* in areas previously conditioned by the same species, the possible existence of a pheromonal attractant, and its bearing on the designs of fungal-preference experiments (See Leonard 1984).

2. Materials and methods

2.1. Collembola

Stock cultures, selection and dispensation of the experimental animals has been previously described (Leonard 1984).

2.2. Evidence of an attractant

Petri dishes, containing 4% water agar (to provide a firm experimental surface), were prepared asceptically. Each plate was then divided into eight equal sections by marking on the base, and in four of these sections, chosen at random, the agar surface was covered with a thin sheet of polythene. The covered areas were designated as unconditioned since the polythene prevented attractants or contaminants, deposited by the Collembola, from being absorbed by the agar. A circle of polythene was also placed in the middle of each plate to provide a surface on which the animals could be added at the start of the experiment, and to prevent contamination of the unconditioned areas by the proximity of conditioned areas at their apex. Approximately 100 animals were then added to each of twenty plates and left overnight.

After this period of conditioning the plates were cleared of Collembola and any exuviae, and the polythene removed. Fifty fresh, test animals were then placed in the middle of each plate and their positions recorded after an hour had elapsed.

2.3. Effects of surface contaminants on aggregation

Twenty agar plates were again prepared and four millipore filters (diameter 25 mm and pore size 0.65 μm) were placed at equal intervals on each plate. Ten Collembola were then located under small plastic containers (which were slightly smaller in diameter than the filters) on two of the four filters. The remaining filters were covered with similar containers without Collembola. The plates were again left overnight.

Following conditioning, the plates were cleared and the filters removed. Thirty fresh Collembola were then placed in the centre of each plate and their positions recorded after one hour had elapsed.

2.4. Effect of volatile substance(s) on aggregation

Twenty plastic dishes, divided into nine equally sized chambers, were filled to within 2 mm of the top with a mixture of plaster of Paris and charcoal. The central chamber was ignored, and groups of ten Collembola were introduced, at random, into four of the remaining eight. Deep lids, with a small central aperture, were sealed across their base with moistened lens tissue. These were placed on top of the divided dishes enclosing the Collembola in their individual chambers. The dishes were left for two hours to allow any volatile substance time to diffuse into the moistened tissue above.

Seventy-five Collembola were then introduced, *via* the aperture in the lid, and allowed to wander freely over the tissue separating them from the Collembola held beneath. The positions of the test Collembola over the eight chambers were recorded directly at hourly intervals under low-level, diffuse light for three hours.

2.5. Potency of the substance(s) versus a food source

Twenty agar plates were prepared and conditioned as described in 2.2. A plug of agar was removed from each of the eight sections, and in the case of two conditioned and unconditioned sections this plug was returned to act as controls. In the remaining four, plugs from a culture of the fungus *Oudemansiella mucida* (Schrader *ex* Fr.) were substituted. This fungus had been shown to be readily palatable to *F. candida* and favoured in choice experiments (J. Lilley, unpublished data).

Fifty fresh animals were placed in the centre of each plate and their positions, with respect to conditioning and the food source, recorded hourly for three hours.

3. Results

3.1. Evidence of an attractant

Counts after one hour had elapsed showed that the Collembola markedly preferred ($P < 0.001$) the conditioned to unconditioned areas, showing a mean count ($\pm$ 1 SE) of 25.0 ($\pm$ 1.30) present on the former sections, whilst only 7.8 ($\pm$ 1.30) were recorded on those areas previously covered with the polythene.

3.2. Effect of surface contaminants on aggregation

Again numbers on conditioned areas were significantly higher ($P < 0.001$). Larger numbers of Collembola (8.8 $\pm$ 1.40) were noted on the areas conditioned through the filters, with only 2.0 ($\pm$ 0.60) occupying the control areas.

3.3. Effect of volatile substance(s) on aggregation

The results obtained after an hour had elapsed were inconclusive. However, by the third hour of monitoring (i.e. five hours after addition of "conditioning" Collembola) the numbers present over the occupied chambers averaged 39.8 ($\pm$ 5.50), proving to be significantly higher than the 13.7 ($\pm$ 3.90) observed on the control areas ($P < 0.01$).

3.4. Potency of the substance(s) versus a food source

Although a palatable food source was present there was still evidence that significantly more ($P < 0.01$) Collembola were attracted to pre-conditioned areas. However, there was no discernable difference between the numbers feeding on the fungal plugs on conditioned and unconditioned areas (Table 1). Very low numbers were recorded on the unconditioned areas without food and, as can be seen in Figure 1, the numbers of Collembola decreased in these areas over the period of observations; animals moving to conditioned areas with food.

4. Discussion

The first experiment showed that areas conditioned by Collembola were attractive to members of the same species. It was possible that the conditioning was due to contamination of the surface with bacterial, fungal or excremental material deposited by the Collembola.

Table 1. Analysis of variance statistics (Sokal & Rohlf 1969), two-way analysis of the numbers of Collembola feeding over time on a fungal food source in conditioned and unconditioned areas

Source of variation	Sum of squares	Degrees of freedom	Mean squares	F. ratio
Between treatments	547.918	3	182.639	3.847*)
Within treatments	81.148	2	40.574	0.855 N.S.
Interaction	7,164.170	6	1,194.030	25.151**)
Residual	10,824.1	228	47.470	
Total	18,617.336	239		

*) $P < 0.01$, **) $P < 0.005$

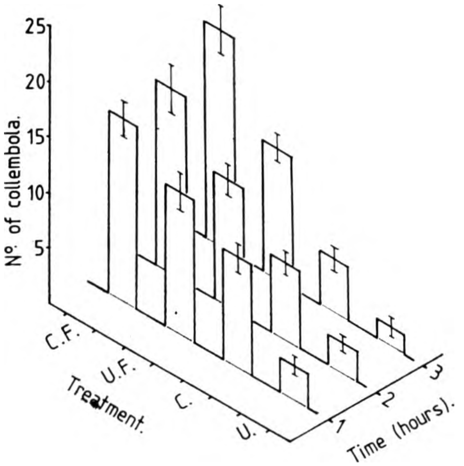

Fig. 1. The numbers of Collembola recorded over three hours present on conditioned and unconditioned areas of the test plates; half of the areas containing a food source. (Mean values ± 1 SE). (U = unconditioned, C = conditioned, F = fungal food source present).

The introduction of a barrier (a millipore filter) between the Collembola and the surface which was conditioned still resulted in aggregation. However, the filter would have allowed the passage of liquids or gaseous products of the Collembola.

Evidence that attractants are volatile, as well as tactile, was found when test animals aggregated over chambers containing other Collembola. No significant aggregations were recorded, however, until the experiment had run for three hours. This suggests that the agent is produced in very small amounts, diffuses slowly, or both.

Leonard (1984) stated that aggregation may influence the results obtained in fungal choice experiments and this must be considered when designing experimental systems. However, these experiments show that there was no difference between the numbers present on the test fungus in conditioned and unconditioned areas, although, as time passed, the numbers on the conditioned areas did significantly increase. As test surfaces in choice experiments will be clean at the start of the experiment, the build-up of an attractant should have little initial influence on the numbers of animals aggregating on favoured diets during investigations of short duration, but this phenomenon must be considered in longer-term experiments as conditioning will cause large aggregations around favoured diets.

Conversely, the effects of locally intense grazing pressures on the fungus must be considered, especially in the field situation were large aggregations of Collembola have been recorded. Despite some conjecture, there is increasing evidence that grazing by Collembola

can have marked affects on decomposition (ADDISON & PARKINSON 1978) and nutrient cycling processes (INESON *et al.* 1982), as well as differential effects on the interactions between litter fungi (VISSER & WHITTAKER 1977) and the balance between the fungal and bacterial populations (HANLON & ANDERSON 1979).

All these effects will be influenced by the density, size and duration of Collembola aggregations in the soil and litter matrix. USHER & HIDER (1975) observed that although the population density of Collembola within an aggregation increased with an increase in the population numbers, this increase was not proportional, suggesting that some Collembola were forced to find or establish new aggregations. Thus the aggregative behaviour of Collembola should be considered an important dynamic process affecting fungal growth and activity in a mosaic of soil and litter microsites.

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7. References

ADDISON, J. A., & D. PARKINSON, 1978. Influence of collembolan feeding activities on soil metabolism at a high arctic site. Oikos **30**, 529—538.

GLASGOW, J. P., 1939. A population study of subterranean soil Collembola. J. Anim. Ecol. **8**, 323—353.

HANLON, R. D. G., & J. M. ANDERSON, 1979. The effect of Collembola grazing on microbial activity in decomposition of litter. Oecologia **38**, 93—99.

INESON, P., M. A. LEONARD & J. M. ANDERSON, 1982. Effect of Collembolan grazing upon nitrogen and cation leaching from decomposing leaf litter. Soil Biol. Biochem. **14**, 601—605.

JOOSSE, E. N. G., 1970. The formation and biological significance of aggregations in the distribution of Collembola. Neth. J. Zool. **20**, 299—314.

LEONARD, M. A., 1984. Observations on the influence of culture conditions on the fungal feeding preference of *Folsomia candida* (Collembola: Isotomidae). Pedobiologia **26**, 361—367.

SOKAL, R. R., & R. J. ROHLF, 1969. Biometry. The principles and practice of statistics in biological research. Freeman, San Francisco.

USHER, M. B., 1969. Some properties of the aggregations of soil arthropods: Collembola. J. Anim. Ecol. **38**, 607—622.

— & HIDER, M., 1975. Studies on populations of *Folsomia candida* (Insecta: Collembola); causes of aggregations. Pedobiologia **15**, 276—283.

VERHOEF, H. A., & C. J. NAGELKERKE, 1977. Formation and ecological significance of aggregations in Collembola. Oecologia **31**, 215—226.

— C. J. NAGELKERKE & E. N. G. JOOSSE, 1977. Aggregation pheromones in Collembola. J. Insect Physiol. **23**, 1009—1013.

VISSER, S., & J. B. WHITTAKER, 1977. Feeding preference for certain litter fungi by *Onychiurus subtenuis* (Collembola). Oikos. **29**, 320—325.

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The presence of the Collembola *Folsomia candida* (WILLEM) on a surface made that area strongly attractive to individuals added later; aggregative behaviour being noted in the conditioned areas. It was shown that solid contaminants were not the causative agent. Further, the substance(s) proved to be volatile. The potency of the attractant and the stability of the resultant aggregation were investigated against a palatable food source. The results being discussed in relation to experimental designs for fungal choice. Possible advantages and problems of aggregative behaviour are discussed.
Key words: Insecta Apterygota, Collembola, Folsomia candida, aggregative behaviour, attractant food, fungi.